

# NATURE OF IRON DEPOSITS ON THE CARDIAC WALLS IN $\beta$ -THALASSEMIA BY MÖSSBAUER SPECTROSCOPY

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## Summary

An identification of the nature and an estimation of the particle size distribution of the iron deposits on thalassemic heart tissue is carried out by variable temperature Mössbauer spectroscopy. Comparison of Mössbauer spectra obtained for the thalassemic heart tissue (I) with those of normal heart tissue (II) and of horse spleen ferritin (III) identifies the iron deposits to be small, superparamagnetic particles of ferritin and/or hemosiderin, two closely related iron storage proteins containing an iron core of  $(\text{FeOOH})_8(\text{FeO} \cdot \text{OPO}_3\text{H}_2)$ . The dependence of the superparamagnetic relaxation time,  $\tau$ , of magnetically ordered fine particles on their volume  $V$  via the magnetic anisotropy constant  $K$  of the material and the condition  $\tau > \tau_L$ , the Larmor precession time of the nuclear magnetic moment of  $^{57}\text{Fe}$  about an effective magnetic field, for observation of hyperfine structure are used in analyzing the Mössbauer data to yield the particle size distribution. Particle diameters are estimated to be  $74 \pm 12 \text{ \AA}$ .

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## Introduction

Thalassemia disorders [1] are accompanied by an excess amount of unbound iron in the red blood cells due to globin chain denaturation and precipitation [2–5,29]. This eventually results in the formation of iron granules [3,5] which are deposited on different internal organs, primarily the spleen, heart, liver, pancreas and lymph nodes [6,7]. Life expectancy is usually 15–20 years and

mortality is most frequently associated with cardiac arrhythmias and congestive heart failure due to myocardial hemosiderosis [8].

Iron-chelation therapy with different agents such as desferrioxamine [9] in the presence of ascorbate [10] which allows the removal and excretion of iron deposits is under investigation, thus offering the possibility of alleviating fatal iron-loading states. The complex chemistry involved in the possible removal of these siderotic deposits in a nontoxic way requires the detailed physicochemical characterization of the state of iron in these deposits. Preferably, the iron should be studied bound on the tissue so that no possible alteration of its physicochemical state is introduced by any chemical reactions that might be involved in an extraction and isolation process of the iron deposits required for an analytical study. Mössbauer spectroscopic studies [11] allow such an investigation and have been used in the detection and characterization of mineral deposits on lungs of coal workers [12] or other occupationally exposed industrial workers [13]. The present work presents a comparative Mössbauer spectroscopic study of the heart tissue obtained at post mortem from two  $\beta$ -thalassemia patients, one male and one female, and from a normal subject. A large amount of iron is detected on the thalassemic heart tissue which is identified via its Mössbauer parameters, superparamagnetic properties and internal magnetic fields as that of the iron storage proteins ferritin and/or hemosiderin \*.

## Experimental

The heart tissues studied were obtained at autopsy from a normal subject and two thalassemic patients, one male and one female. Both thalassemia patients suffered from homozygous  $\beta$ -thalassemia and died at age 18 years.

During his lifetime the male patient had been transfused with nearly 290 units of blood (an iron load of approx. 51 g). Clinical manifestations of heart disease first appeared at 17 years of age and death resulted from intractable congestive heart failure and atrial flutter-fibrillation resistant to therapy. Treatment included digoxin, quinidine, desferrioxamine, folate and vitamin K. At autopsy, the heart weighed 378 g, and all chambers were markedly dilated. There was severe cardiac hemosiderosis, acute multifocal myocardial degeneration, focal fibrosis of papillary muscles of the left ventricle and some calcification of the myocardium and endocardium. Severe hemosiderosis was also present in the liver, lungs and kidneys.

The female patient had received an estimated 350 units of blood transfusions (61 g iron) during her lifetime. Onset of congestive heart failure and angina was at age 13 years, diabetes mellitus requiring insulin developed at age 14 years, and intermittent atrial flutter-fibrillation began at age 17 years. Death was the result of intractable congestive heart failure arrhythmias and renal failure. At autopsy, the heart weighed 466 g. There was marked dilation of all cardiac chambers. Histologic examination disclosed marked and diffuse cardiac hemosiderosis and small, healed infarcts of the posterior papillary muscles on the left

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\* A brief account of this work was presented at the 23rd Annual Conference on Magnetism and Materials (1978) [14].

and anterior papillary muscles on the right. Severe hemosiderosis was extensive in other organs including the liver, pancreas, adrenal glands, kidneys, gastric mucosa, bone marrow, and choroid plexus.

Mössbauer measurements were made at various temperatures between 4.2 and 77 K. A conventional constant acceleration Mössbauer spectrometer operating in the time mode was used, with a  $^{57}\text{Co}$  on Rh source which was held at room temperature. A Teflon sample holder, 1 cm thick and 1 cm diameter, was used, filled with heart tissue material. The sample was mounted in a copper block at the bottom of the cold finger of a liquid helium Dewar flask. Vacuum grease loaded with copper dust was used for good thermal contact of the sample with the cold finger. Temperature variation between 4.2 and 77 K was achieved by introducing a Teflon spacer between the copper block and the cold finger and using a heater wire wrapped around the copper block. Heater regulation and temperature measurement were made with a carbon-glass thermometer mounted in the block.

The Mössbauer results of the thalassemic heart tissues for the two patients were identical and are, therefore, referred to as thalassemic heart tissue (I) and compared with results obtained for normal heart tissue (II). In addition, Mössbauer spectra of horse spleen ferritin (III) were studied for comparison. Ferritin was obtained commercially in a water solution of protein concentration of 100 mg/ml.

## Analysis and Results

The Mössbauer spectra obtained at 77 K for the heart tissue of the normal subject are compared in Fig. 1 with those of the  $\beta$ -thalassemia patients. Strikingly different amounts of iron are contained on the cardiac walls in the two cases as manifested by the intensity of the Mössbauer resonances observed. A more than 100-fold stronger effect is obtained for the thalassemic heart tissue. A least-squares fit to Lorentzian lines for the latter gives an isomer shift  $\delta = 0.45 \pm 0.01$  mm/s relative to iron metal and a quadrupole splitting  $\Delta E_Q = 0.70 \pm 0.01$  mm/s. Thus the  $^{57}\text{Fe}$  resonance is easily observed in the thalassemic heart tissue and a quantitative analysis of the amount of iron deposited due to the disease can be carried out.

In Fig. 1c the Mössbauer spectra of the iron storage protein ferritin at  $T = 77$  K are shown. A least-squares fit analysis to Lorentzian lines for the doublet gives  $\delta = 0.477 \pm 0.007$  mm/s and  $\Delta E_Q = 0.70 \pm 0.01$  mm/s, in good agreement with previously published values by independent investigators [15]. The similarity between the spectra in Figs. 1b and c suggests that the iron deposits on the thalassemic heart wall are in the form of the iron storage protein ferritin or some ferritin-like compound such as hemosiderin [16–19].

To characterize further the nature of the iron deposits, Mössbauer spectra of the thalassemic heart tissue were obtained over a temperature range of 4.2–77 K. Spectra at selected temperatures are shown in Fig. 2. At temperatures below 77 K one observes magnetic hyperfine structure indicative of magnetic ordering or paramagnetic relaxation slower than the  $^{57}\text{Fe}$  Larmor precession time. At low temperatures the spectrum is dominated by a six-line magnetic hyperfine spectrum of an overall splitting of approx. 15.6 mm/s corresponding

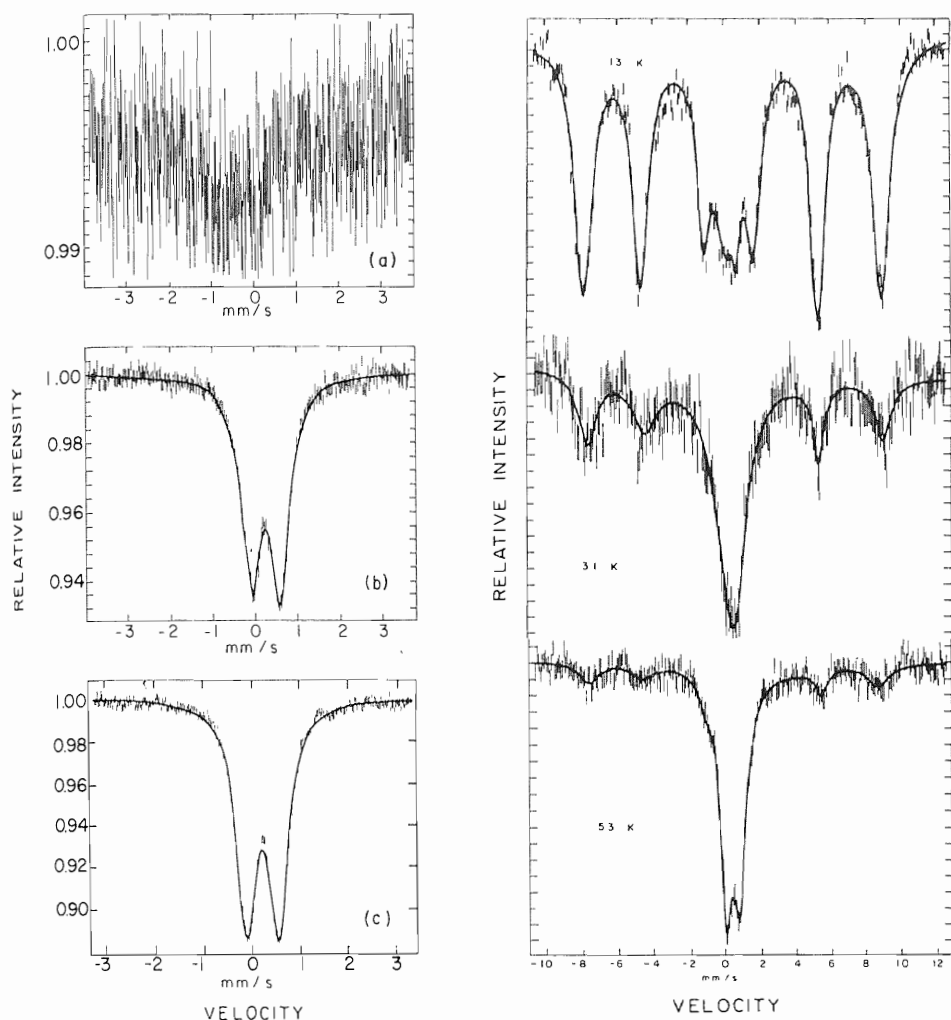


Fig. 1. Mössbauer spectra at  $T = 77$  K of (a) normal heart tissue, (b) thalassemic heart tissue and (c) horse spleen ferritin. The source is  $^{57}\text{Co}$  in Rh at room temperature. Isomer shifts are relative to metallic iron. The solid line in (b) and (c) gives the least-squares fit of the experimental data to Lorentzian lines.

Fig. 2. Mössbauer spectra of thalassemic heart tissue at  $T = 13, 31$  and  $53$  K. The source is  $^{57}\text{Co}$  in Rh at room temperature. Isomer shifts are relative to metallic iron. The solid lines give the least-squares fits of the experimental points to Lorentzian lines.

to an internal field at the nucleus  $H_{\text{eff}}$  approximately equal to 485 kOe. In addition, a quadrupolar split component superimposed at the center of the spectrum is observed. The origin of the central doublet must be attributed to either incomplete spin-alignment at these temperatures or the presence of an additional nonmagnetic iron compound in the tissue. As the temperature increases, the intensity of the hyperfine lines decreases while the central non-magnetically split component builds up in intensity. For  $T > 53$  K only the central quadrupole doublet is observed. Table I summarizes the Mössbauer results.

The magnetic behavior observed above is similar to that obtained by other

TABLE I  
MÖSSBAUER DATA

Sample	Sample temp. (K)	Isomer shift * (mm/s)	Quadrupole splitting (mm/s)	Magnetic hyperfine splitting (mm/s)	$H_{\text{eff}}$ (kOe)
Horse spleen ferritin	4	$0.477 \pm 0.007$	$0.70 \pm 0.01$	$15.63 \pm 0.05$	$484 \pm 2$
	77				
Thalassemic heart tissue	4	$0.45 \pm 0.01$	$0.70 \pm 0.01$	$15.59 \pm 0.09$	$482 \pm 3$
	77				

\* Relative to metallic iron. Source was at room temperature.

investigators [15,18] for ferritin and hemosiderin, in support of the interpretation that the iron deposits in the heart of the thalassemia patients are in the form of ferritin and/or hemosiderin, two closely related iron storage proteins containing an iron core of  $(\text{FeOOH})_8(\text{FeO} \cdot \text{OPO}_3\text{H}_2)$  [20,21]. Furthermore, the temperature dependent hyperfine collapse observed in the present investigation identifies the deposits to be small superparamagnetic particles of these compounds [15,18,21]. The internal field at the iron nucleus depends on the number of unpaired electrons surrounding the nucleus. Whether the internal magnetic hyperfine field splitting is observable by Mössbauer spectroscopy depends on the relaxation time of the unpaired electron spins. If this relaxation time is much longer than the Larmor precession time of the nuclear spin, a magnetically split six-line spectrum is observed. If the relaxation time is shorter than this characteristic time, the average effective field observed at the nucleus is zero and no magnetic structure is observed in the Mössbauer spectrum. For materials with strong antiferromagnetic interactions, such as FeOOH, and for temperatures less than the Néel temperature there exists a critical particle size above which the material exists in the antiferromagnetic state. When fine particles of sizes smaller than the critical size are considered at the same temperature, thermal energy can excite relaxation of the antiferromagnetically ordered spins to the opposite configuration ( $\uparrow\uparrow\uparrow\downarrow \dots \rightleftharpoons \downarrow\uparrow\uparrow\uparrow \dots$ ) which is energetically equivalent. This phenomenon is known as superparamagnetism [23,25]. The relaxation time which measures how rapidly the transition between the two configurations occurs is given by [23]

$$\tau = \tau_0 \exp(2KV/k_B T) \quad (1)$$

where  $K$  is the magnetic anisotropy constant, and  $2K$  the energy required to go from one easy direction of magnetization to the other,  $V$  is the volume of the particle,  $k_B$  is the Boltzmann constant and  $T$  is the temperature.  $\tau_0$  is a temperature independent constant that can be written [22] as  $\tau_0 = 1/af$  where  $a$  is a geometrical factor giving the number of different directions the magnetization vector can flip and  $f$  is the Larmor precession frequency of the magnetization vector about an effective field. Assuming  $a = 2$  [22] and using the value of  $K = 1 \cdot 10^4$  erg/cm<sup>3</sup> for FeOOH [25] and the value of  $f$  obtained by Kündig et al. [22] we get an estimate of  $\tau_0 = 6.6 \cdot 10^{-9}$  s. If we take  $\tau = \tau_L$ , the Larmor precession time of the iron nucleus, the condition for the disappearance of the

magnetic hyperfine lines we may solve Eqn. 1 for the particle volume:

$$V = \frac{k_B T}{2K} \ln(\tau_L / \tau_0) . \quad (2)$$

For the  $^{57}\text{Fe}$  nucleus in a typical field of 500 kOe, the Larmor precession frequency is given by

$$\nu_L = \left( \frac{g_n \mu_n}{\hbar} \right) H_{\text{eff}} \approx 3.9 \cdot 10^7 \text{ s}^{-1} \quad (3)$$

where  $g_n$  is the nuclear  $g$ -factor of the first excited state of  $^{57}\text{Fe}$ ,  $\mu_n$  is the nuclear magneton and  $\hbar$  is the Planck constant. The first excited state of the nucleus is considered here since its  $g$ -factor is smaller than that of the ground state. This gives  $\tau_L$  approximately equal to  $2.5 \cdot 10^{-8}$  s, about a sixth of the Mössbauer measuring time, i.e., the lifetime of the first excited state of  $^{57}\text{Fe}$ . Using the above estimates of the different parameters appearing in Eqn. 2 we may obtain an estimate of the size of the particles observed. The smallest particle volumes would correspond to the temperature at which the hyperfine lines begin to disappear, i.e., approx. 13 K, and the largest particle volumes to the temperature at which the hyperfine lines are almost completely gone, i.e., approx. 60 K. This gives particle volumes of  $8.4 \cdot 10^{-20}$  to  $3.9 \cdot 10^{-19} \text{ cm}^3$ , or particle diameters of approx. 54–90 Å.

The distribution of particle volumes can be obtained from the measurement at various temperatures of the fraction  $f(T)$  of particles with volume greater than the critical volume for superparamagnetism [22–25]. This fraction is just the ratio of the integrated intensity in the magnetic hyperfine spectrum divided by the total intensity, assuming the Debye-Waller factors are equal. This is shown for I in Fig. 3 where  $f$  is plotted vs  $T$ . The solid line is an eye fit to the data. Assuming a particle volume distribution  $n(V)$ ,  $f(T)$  is given by

$$f(T) = \int_{V(T)}^{\infty} n(V') dV' / \int_0^{\infty} n(V') dV' = 1 - \frac{1}{N} \int_0^{V(T)} n(V') dV' \quad (4)$$

where  $V(T)$  corresponds to the critical particle volume for superparamagnetic behavior at temperature  $T$ , and  $N$  is the total number of particles present.

Since  $V$  and  $T$  are interdependent via Eqn. 2 we can equivalently consider  $f$  as a function of the particle volume  $V$  ( $f(T) \Rightarrow f(V)$ ). Then from Eqn. 4 we conclude that the particle volume distribution is proportional to the negative volume derivative of  $f$ :

$$n(V) \propto - \frac{df(V)}{dV}$$

$n(V)$  is shown by the dashed line in Fig. 3 obtained from a graphical evaluation of the slope of the  $f(V)$  vs.  $V$  curve at different values of  $V$ . It is seen that the distribution peaks at approx. 75 Å with a half width at half maximum of 12 Å. Considering the approximate nature of the parameters chosen for Eqn. 2, the result is in good agreement with the values of approx. 50–70 Å observed in ferritin particles by Fischback et al. [16].

Notice that the extrapolated value of  $f(T)$  does not approach unity as  $T$

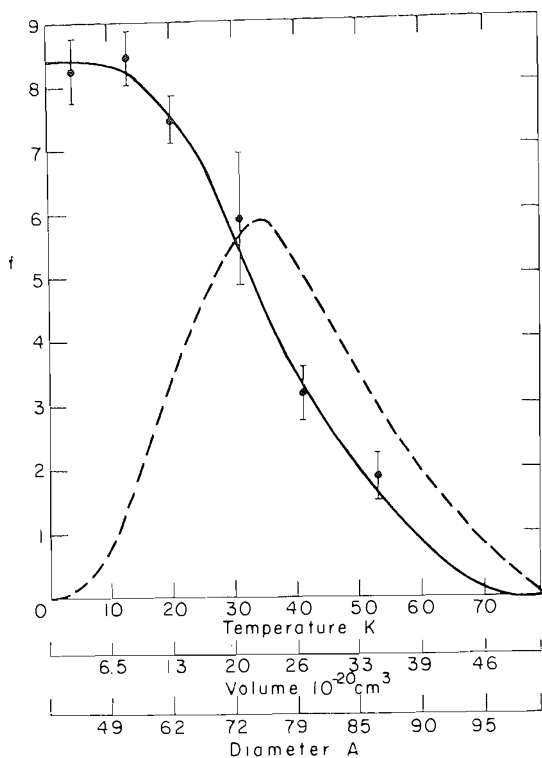


Fig. 3. The fraction  $f$  of particles with volume greater than the critical volume for superparamagnetism given by the ratio of the integrated intensity of the magnetic hyperfine component of the spectrum over the total intensity as a function of temperature or, equivalently, volume or diameter of particles. The solid line gives an eye fit to the experimental points. The dashed line is proportional to  $n(V)$  the particle volume distribution in the sample.

approaches zero. As noted above, this may indicate the presence of another, as yet unidentified, paramagnetic iron compound on the thalassemic heart tissue [26,27].

## Discussion

Most of the iron contained in the body is continuously recycled. About 70% of the iron is bound to hemoglobin and about 20–25% is bound to storage proteins. Ferritin and hemosiderin are the two known forms of iron storage proteins. They both contain a micellar core of  $(\text{FeOOH})_8(\text{FeO} \cdot \text{OPO}_3\text{H}_2)$  [19,20,28] of approx. 70 Å diameter surrounded by a protein shell with a total diameter of approx. 120 Å. Hemosiderin is known to be 24–45% iron by weight while ferritin can vary from zero iron content (apoferritin) to about 23% iron by weight. Thus, it is believed that hemosiderin may be formed when an excess amount of iron is to be stored over and above the available synthesis of the protein shell, i.e., apoferritin.

While the ultimate cure of thalassemia will theoretically lie in alterations of the genes responsible for hemoglobin and possibly apoferritin synthesis, the only present hope of alleviation of the symptoms of the accompanying iron storage disease with acute cardiac problems lies in the continuous development

of better nontoxic chelating agents that will allow the continuous removal of iron deposits from the cardiac walls and other organs.

## Conclusion

We have shown that iron deposited in heart tissue because of thalassemia is easily observable by Mössbauer spectroscopy. We have identified these deposits as hemosiderin or ferritin, and from the temperature dependence of the magnetic hyperfine spectrum, have estimated the size of the particle diameters to be  $74 \pm 12 \text{ \AA}$ .

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